Plant Physiol. (1984) 76, 321-325
0032-0889/84/76/0321/05/$01.00/0

Early Effects of Salinity on Nitrate Assimilation in Barley Seedlings[1]

Received for publication December 28, 1983 and in revised form June 14, 1984

MUHAMMAD ASLAM, RAY C. HUFFAKER,* AND D. WILLIAM RAINS
Plant Growth Laboratory and Department of Agronomy and Range Science, University of California, Davis, California 95616

ABSTRACT

The effect of NaCl and Na_2SO_4 salinity on NO_3^- assimilation in young barley (*Hordeum vulgare* L. var Numar) seedlings was studied. The induction of the NO_3^- transporter was affected very little; the major effect of the salts was on its activity. Both Cl^- and SO_4^{2-} salts severely inhibited uptake of NO_3^-. When compared on the basis of osmolality of the uptake solutions, Cl^- salts were more inhibitory (15–30%) than SO_4^{2-} salts. At equal concentrations, SO_4^{2-} salts inhibited NO_3^- uptake 30 to 40% more than did Cl^- salts. The absolute concentrations of each ion seemed more important as inhibitors of NO_3^- uptake than did the osmolality of the uptake solutions. Both K^+ and Na^+ salts inhibited NO_3^- uptake similarly; hence, the process seemed more sensitive to anionic salinity than to cationic salinity.

Unlike NO_3^- uptake, NO_3^- reduction was not affected by salinity in short-term studies (12 hours). The rate of reduction of endogenous NO_3^- in leaves of seedlings grown on NaCl for 8 days decreased only 25%. Nitrate reductase activity in the salt-treated leaves also decreased 20% but its activity, determined either *in vitro* or by the 'anaerobic' *in vivo* assay, was always greater than the actual *in situ* rate of NO_3^- reduction. When salts were added to the assay medium, the *in vitro* enzymic activity was severely inhibited; whereas the anaerobic *in vivo* nitrate reductase activity was affected only slightly. These results indicate that *in situ* nitrate reductase activity is protected from salt injury. The susceptibility to injury of the NO_3^- transporter, rather than that of the NO_3^- reduction system, may be a critical factor to plant survival during salt stress.

The assimilation of NO_3^-, the predominant form of N available in an aerobic environment, is critical if plants are to adapt, grow, and reproduce in saline conditions. Not only is NO_3^- assimilation required for growth and development, but some of its metabolites accumulate during stress (11, 16, 30). It is well known that both proline (11, 16, 30) and betaine (10) accumulate during stress. Proline apparently originates from recently formed glutamate (5). Methylated quaternary ammonium compounds and possibly some amino acids accumulating in stressed plants could serve as osmotica for osmoregulation (10, 16, 30). Whether or not the N compounds originating from NO_3^- accumulate as symptoms of stress or are osmoregulants, they represent a component of the N economy of the plant and emphasize the need to characterize NO_3^- assimilation during environmental stresses.

Controversial results have been reported for the effects of salinity on N assimilation. Helal *et al.* (12) observed that in spring barley salinization with NaCl impaired growth and uptake of labeled N. The incorporation of labeled N into the protein fraction, however, was increased by salinity. Langdale *et al.* (20) also observed that NaCl salinity increased the protein content of Stargrass. In contrast, NaCl salinization had little effect on N uptake in winter barley but impaired its incorporation into the protein fraction (13). In leaf discs of *Nicotiana rustica*, salt stress reduced both the uptake of L-leucine and its incorporation into proteins (3).

The reported effects of salinity on N assimilation are controversial, because no studies were done that measured all of the processes of NO_3^- assimilation simultaneously. Measuring only uptake or internal reduced N does not yield a balance sheet needed to determine which processes are affected.

This report describes the effects of salinity on the processes of NO_3^- assimilation.

MATERIALS AND METHODS

Seedling Growth. Two varieties of barley (*Hordeum vulgare* L.), Numar and Arivat, were grown. Numar is a salt-tolerant and Arivat is a salt-sensitive variety (7). Seedlings were grown both hydroponically and in vermiculite. For growing seedlings hydroponically, the seeds were germinated in 0.2 mM $CaSO_4$ in darkness at room temperature as described before (1). After 7 d, the seedlings were transferred to aerated one-quarter strength Hoagland solution lacking N (14) and placed in continuous light for 3 d at 25°C and 60% to 65% RH. Photon flux density (400–700 nm) at the seedling canopy was 400 $\mu E\ m^{-2}\ s^{-1}$ and was supplied by incandescent and cool white fluorescent lamps. In some experiments, the seedlings after 2 d in continuous light were transferred to nutrient solutions containing 1 mM KNO_3 (preinduced seedlings) or 0.1 to 0.2 M NaCl or Na_2SO_4 solutions (prestressed seedlings) for 24 h.

For growing seedlings in vermiculite, about 15 g of seeds were planted at a depth of 2 cm in plastic pots (13.5 cm in diameter × 15.0 cm tall) containing vermiculite. The pots were subirrigated with a modified Hoagland solution containing 10 mM KNO_3 or 20 mM KNO_3 and 0.2 M NaCl. In the salt treatment, the seeds were soaked in H_2O for 24 h prior to planting in vermiculite in order to enhance the germination process. The seedlings were grown for 7 to 8 d in a controlled environment growth chamber under a 16-h photoperiod at 65% to 70% RH and 25°/15°C light/dark temperature.

Nitrate Uptake. Uptake of NO_3^- by the whole seedlings grown hydroponically was measured by following its disappearance from the uptake solutions as described before (1, 6). Eight seedlings, weighing about 2 g/treatment, were placed in 140 ml of the uptake solutions. The uptake solutions contained 1 mM KNO_3 and 0 to 0.2 M of either NaCl or Na_2SO_4 in one-quarter strength Hoagland solution. The seedlings prestressed with salts were placed in uptake solutions containing 1 mM KNO_3 only. Cumulative NO_3^- uptake was determined at 2-h intervals in light

[1] Supported in part by a grant from the Kearney Foundation of Soil Science.

(400 μE m^{-2} s^{-1}) at 25°C over a 12-h time course. Rates of NO_3^- uptake were calculated from a linear regression of these curves. At the end of the uptake period, the seedlings were weighed, and analyzed for NO_3^- and NO_2^-. All the data are presented on a g fresh weight basis of plant material.

***In Vivo* Reduction of Absorbed NO_3^-.** *In vivo* reduction of absorbed NO_3^- was determined simultaneously along with uptake. The difference between the total amount of NO_3^- absorbed and that accumulated in the seedlings was considered to be reduced *in vivo* (1, 6). Rates of NO_3^- reduction were calculated from a linear regression of the 12-h time course curves after the linearity was attained.

***In Vivo* Reduction of Endogenous NO_3^-.** The apical 8 cm of leaves from 8-d-old seedlings grown in vermiculite and irrigated with nutrient solutions containing NO_3^- were excised and placed base down in glass vials containing 10 ml of deionized H_2O. The depth of the water in the vials was such that only basal 1.5 cm of the leaves was in water, and the remainder of the leaves were exposed to the atmosphere. The vials containing the leaves were then placed in light (400 μE m^{-2} s^{-1}) at 25°C. The leaves were removed at intervals and analyzed for NO_3^-. No NO_3^- leaked into the water from the leaves during the experimental period. The disappearance of NO_3^- from the leaves is designated as *in vivo* reduction of endogenous NO_3^-.

NR[2] Assay. Nitrate reductase was assayed by both *in vitro* and 'anaerobic' *in vivo* assay methods. For the *in vitro* assay, one g of leaves was homogenized in 4 ml of 0.1 M K-phosphate (pH 7.5) containing 1 mM EDTA, 1 mM cysteine, and 3% (w/v) casein. The homogenate was centrifuged at 30,000*g* for 15 min. The supernatant was used for the enzyme assay. The reaction mixture contained 30 μmol K-phosphate (pH 7.5), 20 μmol KNO_3, 0.5 μmol NADH, 0.1 ml of the supernatant, and various concentrations of NaCl in a total volume of 2 ml. The reaction was carried out at 28°C for 15 min and was stopped by boiling the mixture for 3 min. Excess NADH was oxidized by adding phenazine methosulfate (15 μmol/ml) to the assay mixtures (25). After 20 min, NO_2^- content of the assay mixtures was determined.

For the *in vivo* assay, approximately 0.3 g of leaf sections (2–3 mm wide) were incubated for 1 h in 10 ml of 0.1 M K-phosphate (pH 7.5) containing 50 mM KNO_3 and 1% (v/v) *n*-propanol under dark anaerobic conditions at 28°C. After incubation, both the assay medium and the tissue were analyzed for NO_2^-. The enzyme activities were calculated as μmol NO_2^- produced/g-fresh weight · h.

Nitrate and Nitrite Analysis. The tissue was ground with a mortar and pestle in 10 volumes of deionized H_2O and centrifuged at 30,000*g* for 15 min. The supernatant was used for NO_3^- and NO_2^- analysis. Nitrate was determined by measuring its absorption at 210 nm following separation by HPLC on a Partisil-10-SAX anion exchange column (29). Nitrite was determined colorimetrically as described by Sanderson and Cocking (24).

Determination of Soluble Protein, Sugars, and Chl. Soluble proteins from the plant extract were assayed using the protein-dye binding procedure as described by Bradford (4). Chl was extracted with 80% (v/v) acetone. Absorbance was read at 663 and 645 nm and Chl contents were calculated according to the method of Kirk (19).

Total soluble sugars were extracted by grinding 1 g of the apical 8 cm of leaves in a mortar and pestle with 80% (v/v) ethanol. The extracts were filtered through Whatman filter paper No. 1, heated to 60° to 65°C to evaporate the ethanol, filtered again, and made to 100 ml volume. Total soluble sugars, after acid hydrolysis (28), were determined using the Somogyi method (22) and are reported as glucose equivalents.

Measurement of Osmolality. Osmolality of the uptake solutions was measured with a vapor pressure osmometer.

Transpiration Measurements. The amount of water transpired by the seedlings was measured gravimetrically and is expressed on the basis of shoot.

RESULTS

Effect of NaCl. The rate of NO_3^- uptake by whole seedlings decreased with increasing salt level, most sharply at concentrations greater than 0.1 M (Fig. 1A). A 14, 54, and 83% decrease in the uptake rate occurred at 0.1, 0.15, and 0.2 M NaCl level, respectively. A typical initial lag period of about 4 h occurred before NO_3^- uptake rates became constant. The presence of salts in the uptake solutions did not affect the lag period. The effect of salts was seen after 2 h of uptake. Since the reduction of NO_3^- is dependent on NO_3^- flux (6), the rate of reduction was similarly decreased by salt (Fig. 1B). Salinity had little effect on the per cent reduction (61–71%) of the NO_3^- that was taken up. Since no NO_2^- was detected, it apparently was efficiently assimilated in both stressed and unstressed plants.

Salt had identical effects on short-term NO_3^- assimilation in both the salt-sensitive variety (Arivat) and the salt-tolerant variety (Numar); hence, the results for Arivat are not shown.

Effect of Na_2SO_4. Increasing Na_2SO_4 concentration in the uptake solutions caused 47% and 73% decrease in uptake rate at 0.1 and 0.15 M, respectively (Fig. 2). The rate of NO_3^- reduction also decreased with increasing salt concentration; however, the per cent reduction of the NO_3^- that was taken up was not affected.

Effect of Preinduction with NO_3^-. NaCl and Na_2SO_4 inhibited

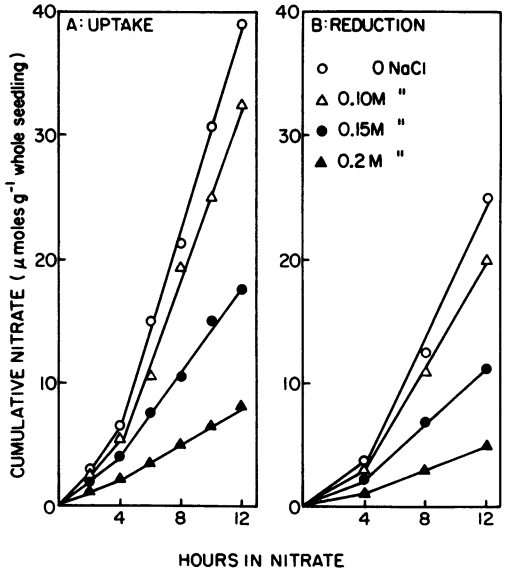

FIG. 1. Effect of NaCl on the time course of NO_3^- uptake and reduction in uninduced Numar barley seedlings. Seedlings were grown hydroponically in NO_3^--free solutions for 7 d in continuous darkness followed by 3 d in continuous light. Nitrate uptake and reduction by the intact seedlings were determined in the presence of 0, 0.10, 0.15, or 0.20 M NaCl in light over a period of 12 h as described in "Materials and Methods". The rates were calculated from a linear regression of the time course curves after the linearity was attained. The rates of NO_3^- uptake (μmol/g·h) were 4.07***, 3.50***, 1.87***, and 0.78*** for 0, 0.10, 0.15, and 0.20 M NaCl, respectively. The corresponding reduction rates (μmol/g·h) were 2.69*, 2.13*, 1.34*, and 0.51*, respectively. The correlation coefficients were significant at 0.05(*) and 0.001(***) probability.

[2] Abbreviations: NR, nitrate reductase; NRA, nitrate reductase activity.

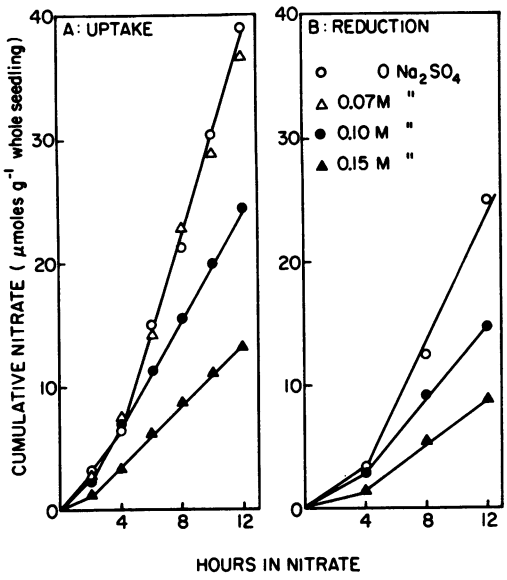

FIG. 2. Effect of Na_2SO_4 on the time course of NO_3^- uptake and reduction in uninduced Numar barley seedlings. Experimental details are the same as in Figure 1 except that the uptake solutions contained various concentrations of Na_2SO_4. The rates of NO_3^- uptake (μmol/g·h) were 4.06***, 3.75***, 2.16***, and 1.11*** for 0, 0.07, 0.10, and 0.15 M Na_2SO_4, respectively. The rates of NO_3^- reduction were 2.69*, 1.41*, and 0.88* for 0, 0.10, and 0.15 M Na_2SO_4, respectively. The correlation coefficients were significant at 0.05 (*) and 0.001 (***) probability.

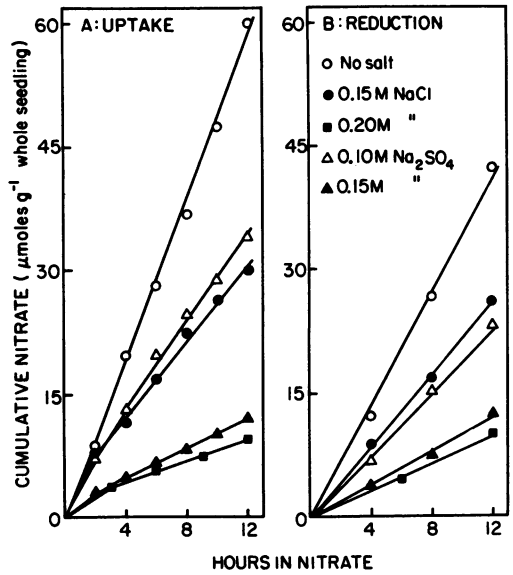

FIG. 3. Effect of NaCl and Na_2SO_4 salts on NO_3^- uptake and reduction in preinduced Numar barley seedlings. The seedlings were grown hydroponically as in Figure 1 except that the seedlings were preinduced with 1 mM KNO_3 for 24 h prior to the study. Other experimental details are the same as in Figure 1. The rates of NO_3^- uptake (μmol/g·h) were 4.99***, 2.22***, 0.73** for 0, 0.15, 0.20 M NaCl, and 2.59*** and 0.92*** for 0.10 and 0.15 M Na_2SO_4, respectively. The corresponding rates of NO_3^- reduction were 3.53**, 2.10**, 0.92*, 1.97**, and 1.08**, respectively. The correlation coefficients were significant at 0.05(*), 0.01(**), and 0.001 (***) probability. Nitrate content of the seedlings at t_0 was 12.6 μmol/g fresh wt.

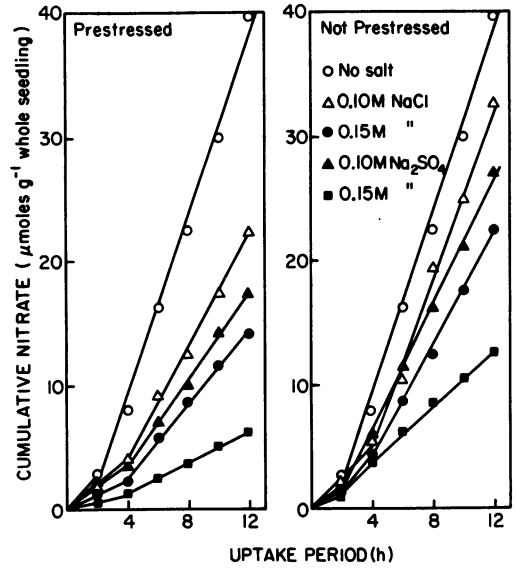

FIG. 4. Comparison of Numar barley seedlings prestressed and not prestressed with NaCl or Na_2SO_4 on subsequent NO_3^- uptake in light. Seedlings were grown hydroponically in NO_3^--free solutions for 7 d in continuous darkness followed by 3 d in continuous light (not prestressed). After 2 d of light, one set of seedlings was transferred to NO_3^--free solutions containing 0.10 or 0.15 M NaCl or Na_2SO_4 for 24 h (prestressed). Nitrate uptake was determined over a period of 12 h as described in "Mateials and Methods". The rates of NO_3^- uptake were calculated from a linear regression of the time course curves after linearity was attained and are presented in Table I.

Table I. *Effect of Salts on Rates of Water Transpiration and NO_3^- Uptake in Prestressed and Not Prestressed Numar Barley Seedlings*

For experimental details, see Figure 4. Correlation coefficients were significant at 0.01 (**) and 0.001 (***) probability.

Salt Concn.	NO_3^- Uptake Rates		H_2O Transpiration Rates	
	Prestressed	Not prestressed	Prestressed	Not prestressed
	μmol/g fresh w·h		*g/g fresh wt·h*	
None	3.88***	3.88***	0.35	0.35
0.10 M NaCl	2.22***	3.50***	0.32	0.33
0.15 M NaCl	1.38***	2.25***	0.25	0.27
0.10 M Na_2SO_4	1.72***	2.52***	0.28	0.29
0.15 M Na_2SO_4	0.55**	1.15***	0.25	0.23

Table II. *Comparative Effect of Cl^- and SO_4^{2-} Salts of Na, at Similar Osmolality, on NO_3^- Uptake Rates in Numar Barley Seedlings*

Experimental details are the same as in Figure 1. Correlation coefficients were significant at 0.001 (***) probability.

Osmolality of Uptake Solution	NO_3^- Uptake Rates		Cl^-/SO_4^{2-}
	NaCl	Na_2SO_4	
mOs	*μmol/g fresh wt·h*		
0	3.73***	3.73***	
200	3.30***	3.66***	0.90
280	1.50***	2.24***	0.67
375	0.79***	0.93***	0.85

NO_3^- uptake similarly in both preinduced and uninduced plants (compare Fig. 3A with Figs. 1A and 2A). As expected, the uptake rates showed no lag in preinduced seedlings. In preinduced seedlings, the proportion of NO_3^- reduced to that taken up

Table III. *Comparative Effect of Na and K Salts of Cl^- and SO_4^{2-} on Rates of NO_3^- Uptake in Numar Barley Seedlings*

Experimental details are the same as in Figure 1. Correlation coefficients were significant at 0.01 (**) and 0.001 (***) probability.

Salt Concn.	NO_3^- Uptake Rates				SO_4^{2-}/Cl^-
	NaCl	KCl	Na_2SO_4	K_2SO_4	
M	*μmol/g fresh wt·h*				
0	3.73***	3.73***	3.73***	3.73***	
0.10	3.30***	3.24***	2.24***	2.20***	0.68
0.15	1.50***	1.50***	0.93***	0.87**	0.60

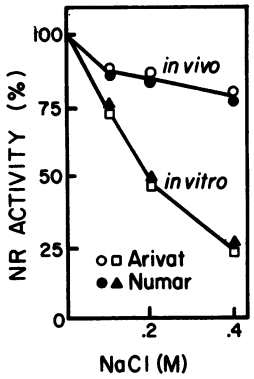

FIG. 5. Effect of NaCl on NRA in primary leaves from salt-sensitive (Arivat) and salt-tolerant (Numar) seedlings. Seedlings were grown for 7 d in vermiculite and irrigated with a full strength Hoagland solution. NRA was assayed in the presence of 0 to 0.4 M NaCl in assay media using both 'anaerobic' *in vivo* and *in vitro* assay methods. The *in vivo* enzyme activities for Numar and Arivat at 100% were 6.8 and 6.2 μmol NO_2^-/g fresh wt·h, and *in vitro* enzyme activities were 23.4 and 19.5 μmol NO_2^-/g fresh wt·h, respectively.

increased with salt concentration (Fig. 3B). At 0.15 M Na_2SO_4 or 0.2 M NaCl, the rates of reduction were greater than the rates of uptake indicating that the higher rate of reduction relied on stored NO_3^-.

Effect of Prestress with Salts. Figure 4 shows the effect of prestress of whole seedlings for 24 h with various levels of salinity on subsequent uptake of NO_3^- from salt-free solutions. Nitrate uptake rates in prestressed seedlings were 35 to 50% lower than those when salts were directly present in the uptake medium (Table I). Prestress of Arivat barley with salt also affected NO_3^- assimilation similarly (data not shown).

Transpiration of water decreased similarly with increased salinity in both the prestressed and not prestressed seedlings (Table I); however, the seedlings appeared healthy, and their water content was not affected over the course of the experiment. Seedling viability was also shown by the ability to reduce their internal NO_3^- (Figs. 1B, 2B, 3B).

Comparative Effect of Cl^- and SO_4^{2-} Salinity. At similar osmolality of uptake solutions, NO_3^- uptake by the seedlings was inhibited more by Cl^- salts than by SO_4^{2-} salts (Table II). However when compared on the basis of salt concentration in the uptake solution, NO_3^- uptake was inhibited more by SO_4^{2-} than by Cl^- salts (Table III). At equimolar concentration, the rates of NO_3^- uptake were 30 to 40% lower in SO_4^{2-} salts than in Cl^- salts. The accompanying cations (Na^+ or K^+) had little effect.

***In Vitro* Effects of Salinity on NRA.** NaCl in the assay medium differentially affected *in vitro* and anaerobic *in vivo* NRA (Fig. 5). Increasing the salinity level in the assay medium decreased the anaerobic *in vivo* NRA only slightly. In contrast, *in vitro* NRA was severely inhibited by salinity. At 0.4 M salinity level, the anaerobic *in vivo* NRA was inhibited only 20% compared with the control, whereas the same level of salinity resulted in an 80% inhibition of *in vitro* NRA. Salinity had a similar effect on enzyme activities in both varieties.

***In Vivo* Effects of Salinity on NO_3^- Assimilation.** Table IV shows the effect of growth for 8 d in NaCl on the levels of NRA in leaves of Numar barley seedlings. Both anaerobic *in vivo* and *in vitro* enzyme activities were 20% lower in leaves of seedlings grown in 0.2 M NaCl salinity as compared with those grown without salt. The rate of reduction of endogenous NO_3^- in stressed leaves also decreased by 25% as compared to that in unstressed leaves (Table IV). The soluble proteins, total soluble sugar, and Chl content of leaves were not affected by salinity (Table IV).

DISCUSSION

The results demonstrate that the processes of NO_3^- assimilation are affected differently by salinity. Uptake of NO_3^- was markedly inhibited, whereas NO_3^- and NO_2^- reduction were little affected by salts (Figs. 1–3).

Present evidence indicates that the NO_3^- transporter is inducible by NO_3^- as shown by a lag period before uptake rate becomes constant (6, 15, 23). Since salinity may affect both the induction and activity of the NO_3^- transporter, the effect of salt was determined on plants both preinduced and uninduced with NO_3^-. No effect of salt was detected on induction of the transporter, and the presence of salts inhibited subsequent uptake in both induced and uninduced plants (Figs. 1A, 2A, and 3A). Thus, salinity affected the activity of the NO_3^- transporter rather than its induction.

Both K and Na salts inhibited NO_3^- uptake similarly (Table III). These results differ from those of Helal *et al.* (13) who observed that addition of KCl relieved the inhibitory effect of NaCl salinity on the uptake of labeled N. In Cl salts, both the anion and the accompanying cation are present in equal proportion; whereas in SO_4^{2-} salts the cationic concentration is twice that of SO_4^{2-}. The differential effect of Cl^- and SO_4^{2-} salts on NO_3^- uptake suggests that the process is more sensitive to anionic salinity than to cationic salinity.

At equal osmolality of solutions, Cl^- salts inhibited NO_3^- uptake from 15% to 30% more than SO_4^{2-} salts (Table II). When compared at the same concentration, SO_4^{2-} salts inhibited NO_3^- uptake more than did Cl^- salts (Table III); hence, the absolute concentrations of each ion seem more important as inhibitors of NO_3^- uptake than does the osmolality of the solutions. Kingsbury *et al.* (18) observed that specific ion effects were more important than was osmotic stress as factors influencing adaptation to salt.

Transpiration of water by the seedlings was inhibited much less by salinity than was NO_3^- uptake; hence, a decreased transpiration rate seemed less responsible for the inhibition of NO_3^- uptake than the specific ion effects (Table I). Previous studies have also shown that a 50% reduction in transpiration had only a minor effect (10%) on NO_3^- uptake (1). Furthermore, in spite of similar transpiration, NO_3^- uptake from nonsaline uptake media by prestressed seedlings was inhibited more than uptake from saline solutions by seedlings not prestressed (Table I; Fig. 4).

When salt was added directly to the assay media, *in vitro* NRA was inhibited much more than was the NRA determined by the anaerobic *in vivo* method using leaf slices (Fig. 5). Schrader (26) also observed similar inhibition of *in vitro* NRA by NH_4^+, K^+, and NaCl salts when added to the assay medium. *In situ* NR in the cytoplasm (27) seems protected against the adverse effects of salinity. Presumably, the salts within the plant cell are compartmentalized into the vacuole (31). There is physiological evidence that in cereals Na^+ is selectively occluded in the vacuole (17).

Reduction of the endogenous NO_3^- in the leaves was decreased

Table IV. *Effect of NaCl on Endogenous NO_3^- Reduction, NRA, Soluble Protein, Soluble Sugars, and Total Chl Content in Primary Leaves of Numar Barley Seedlings*

Seedlings were grown in vermiculite for 8 d and irrigated with nutrient solutions containing 0 and 0.2 M NaCl and 10 (at 0 NaCl) or 20 mM (at 0.2 M NaCl) KNO_3. *In vivo* reduction of endogenous NO_3^- was determined by following the disappearance of NO_3^- from the leaves in light as described in "Materials and Methods". The rates of NO_3^- reduction were calculated from the time course curves which were linear up to 12 h.

NaCl Level	NRA		Endogenous NO_3^- Concn. at t_0	Rate of Endogenous NO_3^- Reduction	Soluble Protein	Chl	Soluble Sugars (Glucose equiv.)
	In vivo	*In vitro*					
M	$\mu mol\ NO_2^-/g \cdot h$		$\mu mol/g$	$\mu mol/g \cdot h$		mg/g	
0	4.7 ± 0.5	18.3 ± 0.8	42.5 ± 4.5	2.16	9.54 ± 0.66	1.61 ± 0.05	6.21 ± 0.55
0.2	3.7 ± 0.4	14.5 ± 0.6	34.4 ± 3.5	1.63	10.15 ± 0.39	1.55 ± 0.04	6.51 ± 0.73

only 25% by salinity. Nitrate reduction was not limited by the potential NRA (*in vitro* or anaerobic *in vivo*), which was greater than the rate of endogenous NO_3^- reduction (Table IV). Since the major portion of NO_3^- is stored in the vacuole (9, 21), especially a few hours after the removal of NO_3^- from the nutrient solution (2, 8), the release of NO_3^- from vacuoles might regulate the rate of NO_3^- reduction (2). Thus, the small decrease in the rate of NO_3^- reduction may be partially due to NaCl inhibiting NO_3^- efflux from vacuoles.

LITERATURE CITED

1. ASLAM M, RC HUFFAKER, DW RAINS, KP RAO 1979 Influence of light and ambient carbon dioxide concentration on nitrate assimilation by intact barley seedlings. Plant Physiol 63: 1205–1209
2. ASLAM M, A OAKS, RC HUFFAKER 1976 Effect of light and glucose on the induction of nitrate reductase and on the distribution of nitrate in etiolated barley leaves. Plant Physiol 58: 588–591
3. BEN-ZIONI A, C ITAI, Y VAADIA 1967 Water and salt stresses, kinetin and protein synthesis in tobacco leaves. Plant Physiol 42: 361–365
4. BRADFORD MM 1976 A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248–254
5. BUHL MB, CR STEWART 1983 Effects of NaCl on proline synthesis and utilization in excised barley leaves. Plant Physiol 72: 664–667
6. CHANTAROTWONG W, RC HUFFAKER, BL MILLER, RC GRANSTEDT 1976 *In vivo* nitrate reduction in relation to nitrate uptake, nitrate content, and *in vitro* nitrate reductase activity in intact barley seedlings. Plant Physiol 57: 519–522
7. EPSTEIN E, JC NORLYN, DW RUSH, RW KINGSBURY, DB KELLEY, GA CUNNINGHAM, AF WRONA 1980 Saline culture of crops: A genetic approach. Science 210: 399–404
8. FERRARI TE, OC YODER, P FILNER 1973 Anaerobic nitrite production by plant cells and tissues: evidence for two nitrate pools. Plant Physiol 51: 423–431
9. GRANSTEDT RC, RC HUFFAKER 1982 Identification of the leaf vacuole as a major nitrate storage pool. Plant Physiol 70: 410–413
10. HANSON AD, CE NELSEN 1978 Betaine accumulation and [^{14}C]formate metabolism in water-stressed barley leaves. Plant Physiol 62: 305–312
11. HANSON AD, CE NELSEN, EH EVERSON 1977 Evaluation of free proline accumulation as an index of drought resistance using two contrasting barley cultivars. Crop Sci 17: 720–726
12. HELAL M, K KOCH, K MENGEL 1975 Effect of salinity and potassium on the uptake of nitrogen and on nitrogen metabolism in young barley plants. Physiol Plant 35: 310–313
13. HELAL HM, K MENGEL 1979 Nitrogen metabolism of young barley plants as affected by NaCl-salinity and potassium. Plant and Soil 51: 457–462
14. HOAGLAND DR, DI ARNON 1950 The water-culture method for growing plants without soil. Calif Agric Exp Stn Cir 347
15. JACKSON WA, D FLESHER, RH HAGEMAN 1973 Nitrate uptake by dark-grown corn seedlings: Some characteristics of apparent induction. Plant Physiol 51: 120–127
16. JEFFERIES RL 1980 The role of organic solutes in osmoregulation in halophytic higher plants. *In* DW Rains, RC Valentine, A Hollaender, eds, Genetic Engineering of Osmoregulation, Basic Life Sciences, Vol 14. Plenum Press, New York, pp 135–154
17. JESCHKE WD 1979 Univalent cation selectivity and compartmentation in cereals. *In* DL Laidman, RG Wyn Jones, eds, Recent Advances in the Biochemistry of Cereals. Academic Press, London, pp 37–62
18. KINGSBURY RW, E EPSTEIN, RW PEARCY 1984 Physiological responses to salinity in selected lines of wheat. Plant Physiol 74: 417–423
19. KIRK JTO 1968 Studies on the dependence of chlorophyll synthesis on protein synthesis in *Euglena gracilis*, together with a nomogram for determination of chlorophyll concentration. Planta 78: 200–207
20. LANGDALE GW, JR THOMAS, TG LITTLETON 1973 Nitrogen metabolism of stargrass as affected by nitrogen and soil salinity. Agron J 65: 468–470
21. MARTINOIA E, U HECK, A WIEMKEN 1981 Vacuoles as storage compartments for nitrate in barley leaves. Nature (Lond) 289: 292–293
22. NELSON N 1944 A photometric adaptation of the Somogyi method for the determination of glucose. J Biol Chem 153: 375–380
23. RAO KP, DW RAINS 1976 Nitrate absorption by barley. I. Kinetics and energetics. Plant Physiol 57: 55–58
24. SANDERSON GW, EC COCKING 1964 Enzymic assimilation of nitrate in tomato plants. I. Reduction of nitrate to nitrite. Plant Physiol 39: 416–422
25. SCHOLL RL, JE HARPER, RH HAGEMAN 1974 Improvements of the nitrite color development in assays of nitrate reductase by phenazine methosulfate and zinc acetate. Plant Physiol 53: 825–828
26. SCHRADER LE 1978 Uptake, accumulation, assimilation and transport of nitrogen in higher plants. *In* DR Nielson, JG MacDonald, eds, Nitrogen in the Environment, Vol II. Academic Press, New York, pp 101–141
27. SCHRADER LE, LE BEEVERS, RH HAGEMAN 1967 Differential effects of chloramphenicol on the induction of nitrate and nitrite reductase in green leaf tissue. Biochem Biophys Res Commun 26: 14–17
28. Smith D 1969 Removing and analyzing total nonstructural carbohydrates from plant tissue. Univ Wis Res Rep 41
29. THAYER JR, RC HUFFAKER 1980 Determination of nitrate and nitrite by high-pressure liquid chromatography: Comparison with other methods for nitrate determination. Anal Biochem 102: 110–119
30. WYN JONES RG 1980 An assessment of quaternary ammonium and related compounds as osmotic effectors in crop plants. *In* DW Rains, RC Valentine, A Hollaender, eds, Genetic Engineering of Osmoregulation, Basic Life Sciences, Vol 14. Plenum Press, New York, pp 155–170
31. WYN JONES RG 1981 Salt tolerance. *In* CB Johnson, ed, Physiological Processes Limiting Plant Productivity. Butterworths, London, pp 271–292